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MORPHOMETRIC ANALYSIS OF UNIPARENTAL *APHYTIS* REARED FROM CHAFF SCALE, *PARLATORIA PERGANDII* COMSTOCK, ON TEXAS CITRUS (HYMENOPTERA: APHELINIDAE; HOMOPTERA: DIASPIDIDAE)

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Abstract.—The literature on parasites of chaff scale, *Parlatoria pergandii* Comstock, is briefly reviewed with emphasis on chaff scale in Texas. A survey of the natural enemies of chaff scale in Texas citrus showed two thelytokous and closely related (cryptic) species, *Aphytis hispanicus* (Mercet) and *Aphytis comperei* DeBach and Rosen, to be the most common parasites. Since these species are reported in the literature to be sympatric in many localities, and since individuals with an apparently intermediate morphology were found, we tested the hypothesis that the concepts of *A. hispanicus* and *A. comperei* represent two points in a continuous distribution of phenotypes. A morphometric study of the material was conducted to determine if two distinct morphs corresponding to *A. hispanicus* and *A. comperei* occur in Texas citrus, and if so, to identify useful morphological characters to distinguish between them. Sixteen measurements of anatomical structures, six meristic characters, and two qualitative characters were scored for 146 specimens reared from isolated chaff scale. The measurement data were analyzed using principal component and canonical variates analyses. Principal component analysis of the raw and log-transformed data showed that two distinct morphs exist which correspond to *A. hispanicus* and *A. comperei.* In addition, a third group of individuals, designated as *A. ?hispanicus,* was found. These individuals are close to, but somewhat distinct from, *A. hispanicus.* Principal component analysis and canonical variates analysis suggest that the *A. ?hispanicus* group consisted of small specimens of *A. hispanicus.* Canonical variates analysis also showed that 6 of the 17 characters used were useful in discriminating between *A. comperei* and *A. hispanicus.* Two meristic characters showed strong discontinuities between *A. comperei* and *A. hispanicus.* We conclude that two species, *A. comperei* and *A. hispanicus,* are the common parasites of chaff scale in Texas citrus.

This paper is the first in a series reporting the results of a survey of the parasites of armored scale on citrus in south Texas. Here we discuss the status of uniparental (thelytokous) *Aphytis* (Hymenoptera: Aphelinidae) reared from chaff scale, *Parlatoria pergandii* Comstock (Homoptera: Diaspididae). Chaff scale is a recurring problem on citrus in Texas (Dean, 1955; Dean et al., 1983), Israel (Gerson, 1977; Harpaz, 1961), Spain (Limon et al., 1977; Carrero, 1980), and in other locations worldwide (Talhouk, 1975) *Parlatoria pergandii* occurs sympatrically in Israel with *Parlatoria cinerea* Doane and Hadden, the tropical grey chaff scale (Gerson, 1967a, b).

The most common parasites we encountered on chaff scale were referable to one of two species in the *proclia* group of *Aphytis*: *A. hispanicus* (Mercet) and *A. comperei* DeBach and Rosen. Both species are uniparental (Rosen and DeBach, 1979) and males referable to this species group were quite rare in our collections. The similarities between these two species and the occurrence of apparent intermediate forms prompted this study.

Prior to the work of DeBach and Rosen (summarized in Rosen and DeBach, 1979) species identification in *Aphytis* was extremely difficult and often controversial. Dean (1965) expressed some frustration at the different species names various specialists provided for *Aphytis* reared from chaff scale on Texas citrus—variously *A. proclia* (Walker), *A. diaspidis* Howard, and *A. hispanicus*. All of these species are in the *proclia* group, as are *A. maculicornis* (Masi) and *A. paramaculicornis* DeBach and Rosen which were imported and released against the olive scale, *Parlatoria oleae* (Colvée), in California and elsewhere (Rosen and DeBach, 1979). Dean (1965), Dean and Hoelscher (1967) and Dean and Bailey (1960) refer to an *Aphytis* "complex" as the dominant parasites of chaff scale in Texas. Later, DeBach and Rosen (1976) determined that Dean's chaff scale parasite material contained two very similar *Aphytis* species: *A. hispanicus* and a second species they described as *A. comperei*.

Our examination of early correspondence and unpublished reports indicate that chaff scale was present early in the history of the citrus industry in Texas. A complete review of all parasites of chaff scale in Texas is forthcoming. Introduction of other *Aphytis* species have been made for chaff scale control in Texas, but establishments of exotic species have not been documented (Dean and Bailey, 1960). In 1968 a strain of *A. paramaculicornis* originally obtained from chaff scale on citrus at Escondido, California was shipped from Riverside to Texas and approximately 35 adults were released on chaff scale at Weslaco. This somewhat enigmatic *Aphytis* was originally thought to be the "Iran" strain of *A. paramaculicornis*, but Rosen and DeBach (1979) regard it as distinct and apparently indigenous to California. No recoveries of this *Aphytis* have been made. In fact, we have not reared material from chaff scale in Texas referable to any introduced species. All *Aphytis* found on chaff scale in Texas can therefore be considered to be indigenous, or exotic species which moved with chaff scale when it was introduced to Texas.

The holotype of *Aphytis hispanicus* Mercet (1912) was from material reared from chaff scale on citrus at Valencia, Spain. The species was most recently redescribed by Rosen and DeBach (1979), whose list of localities for this species includes Spain, Italy, Turkey, Israel, the Caucasus, Morocco, Taiwan, Brazil, Trinidad, Mexico, Florida, California, and notably, Texas. Most of the specimens were reportedly reared from *P. pergandii*, and to a less extent from *P. cinerea, P. oleae, Aspidiotus nerii* Bouché, *Insulaspis pallida* (Green), *Lopholeucaspis japonica* (Cockerell), and *Mytilaspis conchiformis* (Gmelin). Records from *Acutaspis scutiformis* (Cockerell), *Aonidiella aurantii* (Maskell) and *Chrysomphalus dictyospermi* (Morgan) were regarded as questionable. *Aphytis hispanicus* has also been reported attacking chaff scale in Morocco (Abassi, 1975), Spain (Limon et al., 1976, 1977) and Florida (Muma, 1971). Crouzel (1973) listed *A. hispanicus* and *Aphytis argentinus* Brèthes as parasites of *P. cinerea* and *P. pergandii*, respectively, in Argentina. However, Gerson (1977) stated that the chaff scale parasite which she listed as *A. argentinus* is probably a synonym of *A. comperei* or *A. hispanicus*. Rosen and DeBach (1979) describe *A. hispanicus* as a uniparental, solitary parasite of *Parlatoria* species which attacks second instars, male scale, and adult

Table 1. Diagnostic characters for *Aphytis comperei* and *A. hispanicus* from Rosen and DeBach (1979). Information taken from key to species and species redescriptions.

	A. hispanicus	*A. comperei*
Genal sutures	heavily sclerotized, infuscate from oral margin to about ¾ distance to eye	less heavily sclerotized, faintly infuscate
Antennal club	length/width = 2.5–3.0	shorter, thicker, length/width about 2.3
Pedicel, funicle, base of club	uniformly and strongly infuscate	paler
Antennal club	apical ⅓ blackish (infuscate)	tip with conspicuous black spot
Crenulae	6–8 per side, elongate, non-overlapping, faintly infuscated	3–5 per side, wider, distinctly blackish
Ratio of lengths of ovipositor/middle tibia	less than or = 1.33	longer, up to 2.00
Forewing, length/width	2.75–3.00	2.5–2.66
Setae in delta region of forewing	59–161 in 9–12 rows	51–96 in 7–9 rows
LMC forewing/width forewing	0.17–0.33	0.20

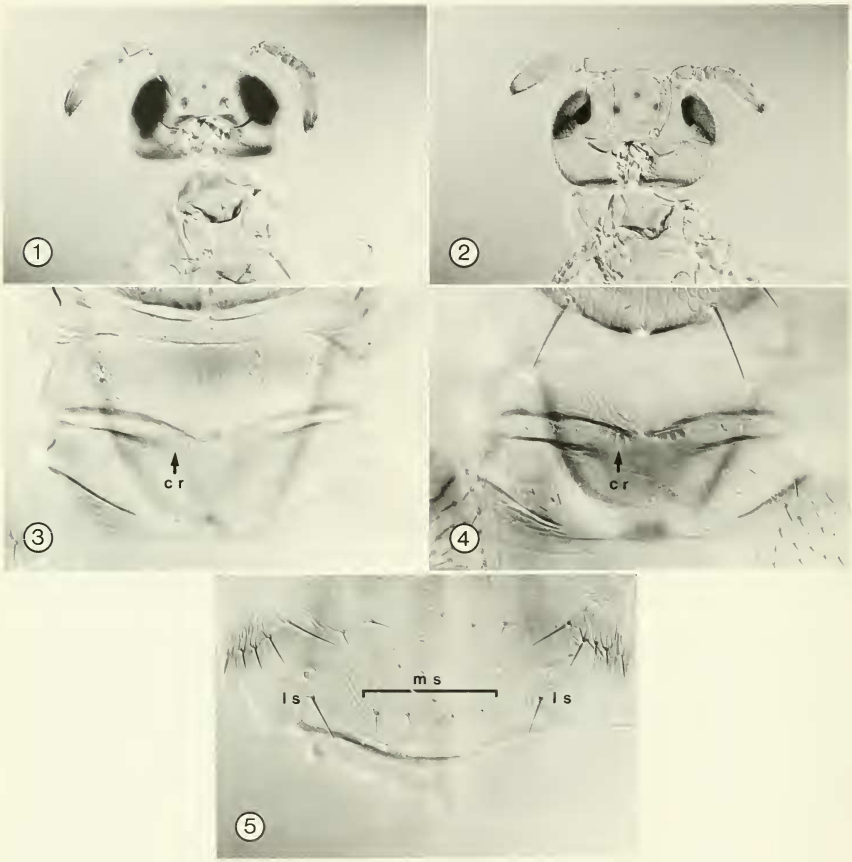
female scale, preferring the latter. Gerson (1967b, 1968), Rosen (1965, 1967, 1969) and Rivnay (1968) provide information on the biology of *A. hispanicus* in Israel.

DeBach and Rosen (1976) described *A. comperei* and provided diagnostic characters to distinguish it from *A. hispanicus* and *A. proclia*. The holotype female was reared from "*Aonidiella aurantii* material" on citrus in McAllen, Texas; however, Rosen and DeBach (1979) regarded the California red scale record for the holotype as questionable. They point out that California red scale, chaff scale, and other scale species are often found mixed together on citrus and cross contamination of rearing samples is common. Most of the records listed for *A. comperei* are from chaff scale, and records from *A. aurantii, Chrysomphalus aonidum* (L.), and *Cornuaspis beckii* (Newman) were regarded as questionable (Rosen and DeBach, 1979). The distribution of *A. comperei* includes Texas, Mexico, Florida, Jamaica, South Africa, Hong Kong and Canton, China. Little additional information is available on the biology of *A. comperei* beyond the observation of Rosen and DeBach (1979) that this species is uniparental.

Rosen and DeBach (1979) provide a number of characters to distinguish *comperei* and *hispanicus* in their key to *Aphytis* species and in the redescriptions of the two species. We list their criteria in Table 1 and provide figures of typical character states for each species. Of particular interest are the shape and coloration of the antennal segments, the conformation of the crenulae, and aspects of the forewings. In the next few paragraphs, all discussion of the diagnostic characters for the two species refers to the criteria of Rosen and DeBach (1979).

The antennal club of *A. hispanicus* (Fig. 1, Table 1) is characterized by a blackish, infuscate region in about the distal third. The antennal club of *A. comperei* (Fig. 2, Table 1) is shorter, thicker, and the infuscate area is confined to a black spot at the tip.

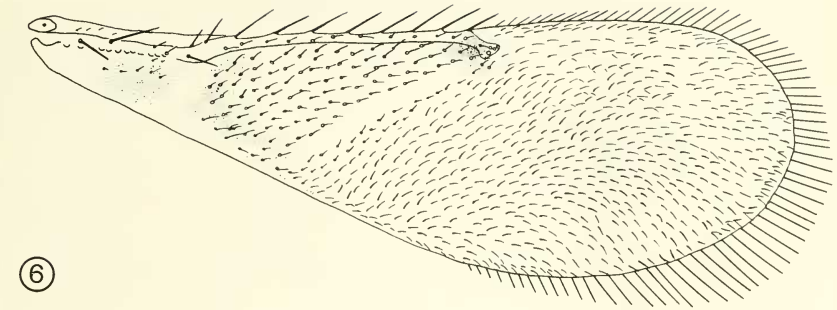
The propodeum of *Aphytis* typically bears several posterior, lamellate projections called crenulae. Variations in the size, shape, number, and color of the crenulae have been used to distinguish between many *Aphytis* species (Rosen and DeBach, 1979). These authors state that 12–16 crenulae are found on *A. hispanicus* (Table 1), and further, that the crenulae in *hispanicus* are elongate, pale

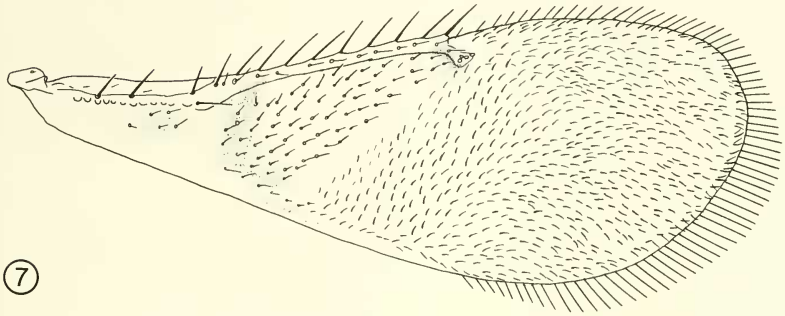


Figs. 1–5. Photomicrographs of *Aphytis* species. 1, Antennae of *A. hispanicus,* medial aspect. 2, Antennae of *A. comperei,* medial aspect. 3, Propodeum of *A. hispanicus.* 4, Propodeum of *A. comperei.* 5, Eighth abdominal tergum of *A. hispanicus.* (cr = crenulae, ls = lateral setae, ms = medial setae).

in color, and do not overlap one another (Fig. 3). In contrast, *A. comperei* is characterized by 6–10 crenulae (Table 1), and the crenulae are wider, overlapping, and distinctly darker in color (Fig. 4).

The forewing of *A. hispanicus* is longer and narrower than that of *A. comperei* (Table 1, Figs. 6 and 7), but the differences are not striking. The oblique, bare streak in the aphelinid wing is properly termed the linea calva (Hayat, 1983). In *Aphytis* the region of the forewing basal to the linea calva is called the delta region (Rosen and DeBach, 1979). As noted in Table 1, specimens of *A. hispanicus* are said to commonly bear more setae (microtrichiae) in the delta region (59–161) than specimens of *A. comperei* (51–96 setae). Finally, the ratio of the longest mar-





Figs. 6, 7. 6, Forewing of *A. hispanicus.* 7, Forewing of *A. comperei.*

ginal cilia (LMC) on the forewing to the greatest width of the forewing in *hispanicus* specimens is usually longer (ratio of 1/6–1/3) than in *comperei* specimens (1/5) (Table 1).

Several of the characters in Table 1 are extremely subtle, and require some subjective interpretation by the observer. The ranges given for several other characters are strongly overlapping (e.g. the number of setae in the delta region of the forewing). Further, some individuals in our material show an intermediate morphology, suggesting that Rosen and DeBach's (1979) concepts of *A. comperei* and *A. hispanicus* might represent two ends of a continuous distribution of phenotypes. The two species are often sympatric (in the sense that both are often reared from the same collection of *Parlatoria* scale) which is consistent with this contention. As both species are uniparental, a purely phenetic species concept is appropriate. Differences in biology, behavior, or ecology, were they known, would support the recognition of two species.

The objective of this study was to determine if two distinct morphs, corresponding to *A. comperei* and *A. hispanicus,* occur in south Texas citrus, and if so, to determine by what morphological attributes they can best be distinguished. The null hypothesis, in a sense, was that the morphs corresponding to these species are not distinct and that they intergrade. We analyzed the variation in a set of morphological characters in our

populations in order to resolve these alternatives.

Methods

Parlatoria pergandii material was collected from 28 citrus groves in Hidalgo and Cameron counties in south Texas during March through November, 1983. Groves were generally unsprayed, and formed an east-west transect of the citrus production region in Texas. Individual parasitized *P. pergandii* were identified by lifting scale covers with a dissecting probe. Individuals were then isolated in 0.25 dram glass shell vials with cotton stoppers. The parasites were allowed to emerge and die in the vials and were then slide-mounted in Hoyer's medium (Rosen and DeBach, 1979). A total of 146 slide-mounts of specimens referable to either *A. comperei* or *hispanicus* were available for study at the time the morphometric analyses were begun.

Data were collected from all available specimens using a Zeiss compound microscope equipped with Nomarski contrast enhancement. Measurements on specimens were taken through either a 16× or 40× objective, using a 12.5× eyepiece containing a reticle with 100 divisions. The eyepiece reticle was calibrated with a stage micrometer allowing conversion of eyepiece reticle units to microns, the scale used for all quantitative measurements.

At the time the data were taken, a tentative species determination was made for each specimen, using the criteria of Rosen and DeBach (1979) (Table 1). In seven cases, the character states of a specimen were outside the stated range for either species, but close to one of them. Five individuals were assigned a tentative determination of *A. ?hispanicus* and two as *A. ?comperei.*

The character set used was a mixture of quantitative (continuous) measurements (Table 2), meristic (counted), and coded multistate (qualitative) characters. The character set was assembled using various criteria. We re-examined the characters of Rosen and DeBach (1979), accounting for characters 1, 4, 5, and 9–15 in Table 2. We also included several additional measurements (characters 2, 3, 6–8, 16 and 17 in Table 2) so that the data would better describe differences in shapes between specimens.

Rosen and DeBach (1979) stressed the value of the crenulae for species discrimination. We coded data for the crenulae as: the number of crenulae (meristic), the color of the crenulae (dark, some dusky color, or pale), and the degree to which the crenulae were overlapping (overlapping, contiguous but not overlapping, or well separated). In addition, we counted the setae present on three abdominal terga: the seventh, lateral setae on the eighth, medial setae on the eighth, and on the syntergum (following Rosen and DeBach's (1979) numbering of terga, in which the propodeum is counted as the first tergum).

All morphometric analyses on the data set were performed using the Statistical Analysis System (SAS) software (SAS Institute, 1982a, b) on a VAX 11/750 microcomputer. Sixteen quantitative characters and one meristic character (the number of setae in the delta region of the forewing) were used for morphometric analyses (Table 2).

Principal components analysis (PCA) was performed on the variance/covariance matrix computed from the raw data. In all multivariate statistical procedures, SAS programs remove any observations with missing data points for variables used in the analysis. Twenty observations had missing data for one or more quantitative characters, leaving 126 observations available for the PCA. Principal components computed from a variance/covariance matrix may be sensitive to the greater variance associated with characters with numerically larger values (Neff and Marcus, 1980). For this reason, PCA was also performed on the variance/covariance matrix computed from the logarithms (base 10) of the raw data.

Table 2. Univariate statistics for variables used in the morphometric analyses. Means, 95% confidence intervals around the means, and ranges are given in microns. Values are lengths, unless otherwise indicated. An asterisk (*) preceding the variable number indicates that the character was discussed by DeBach and Rosen (1976) and/or Rosen and DeBach (1979).

Variable	*A. comperei* $\bar{x} \pm$ 95% CI, n Range	*A. hispanicus* $\bar{x} \pm$ 95% CI, n Range	*A. ?hispanicus* $\bar{x} \pm$ 95% CI, n Range
*1) Scape	96.0 ± 1.46, 100 72.3 to 109.5	97.8 ± 2.41, 37 78.8 to 111.7	81.5 ± 6.91, 5 72.3 to 92.0
2) Pedicel	37.2 ± 0.57, 101 28.5 to 43.8	36.0 ± 1.58, 38 12.0 to 41.6	31.1 ± 2.58, 5 26.3 to 32.8
3) Apical funicle segment	34.1 ± 0.56, 102 26.3 to 39.4	33.4 ± 0.72, 37 26.3 to 37.2	25.8 ± 1.65, 5 24.1 to 28.5
*4) Antennal club	78.8 ± 1.31, 98 61.3 to 94.2	82.3 ± 1.92, 37 67.9 to 96.4	68.6 ± 1.92, 5 65.7 to 70.1
*5) Infuscation on antennal club	20.2 ± 0.64, 97 13.1 to 28.5	35.3 ± 1.35, 37 28.5 to 43.8	28.9 ± 3.25, 5 26.3 to 35.0
6) Mesoscutum	98.7 ± 1.76, 101 74.5 to 116.1	99.8 ± 2.36, 36 85.4 to 118.3	81.9 ± 4.97, 5 76.6 to 89.8
7) Scutellum	80.9 ± 1.52, 102 59.1 to 94.2	83.6 ± 1.98, 37 65.7 to 92.0	67.9 ± 7.79, 5 59.1 to 81.0
8) Metanotum	13.6 ± 0.30, 103 11.0 to 17.5	15.0 ± 0.51, 38 13.1 to 17.5	12.7 ± 1.65, 5 11.0 to 15.3
*9) Propodeum	50.3 ± 0.98, 103 37.2 to 61.3	58.1 ± 1.60, 38 48.2 to 65.7	45.1 ± 7.62, 5 35.0 to 56.9
*10) Ovipositor	284.3 ± 4.01, 103 224.4 to 325.4	261.3 ± 4.35, 38 235.6 to 291.7	225.5 ± 15.79, 5 207.6 to 252.4
*11) Forewing (length)	553.0 ± 8.51, 102 415.1 to 628.3	572.2 ± 10.54, 37 493.7 to 645.2	471.2 ± 34.94, 5 432.0 to 527.3
*12) Forewing (width)	201.2 ± 3.73, 101 140.2 to 241.2	192.7 ± 4.13, 37 162.7 to 218.8	158.2 ± 12.62, 5 145.9 to 179.5
*13) LMC on forewing	38.7 ± 1.06, 102 28.0 to 56.1	50.6 ± 2.13, 37 33.7 to 61.7	57.2 ± 8.33, 5 44.9 to 67.3
*14) Setae in delta region, forewing	67.4 ± 2.09, 100 37 to 89	99.5 ± 4.64, 37 73 to 129	62.6 ± 11.88, 5 50 to 80
*15) Middle tibia	152.3 ± 2.57, 101 116.1 to 175.2	163.4 ± 3.49, 38 138.0 to 179.6	131.8 ± 11.99, 5 118.3 to 151.1
16) Basitarsus	51.0 ± 1.12, 103 35.0 to 63.5	56.7 ± 1.62, 38 46.0 to 67.9	42.9 ± 6.94, 5 35.0 to 54.8
17) Apical spur, middle tibia	50.4 ± 0.82, 103 37.2 to 59.1	56.4 ± 1.35, 38 46.0 to 65.7	45.6 ± 3.25, 5 41.6 to 50.4

Canonical variates analysis (CVA) was used to further evaluate particular variables for species discrimination. As discussed below, the original species determinations were supported (in most cases) by the PCA, therefore, these determinations were used as the class variable for CVA. We expected that the first canonical variate would be constructed to optimally discriminate *A. hispanicus* and *A. comperei* given the presence of the third class of *A. ?hispanicus* individuals. In addition, given the hypothesis that the *A. ?hispanicus* individuals were somewhat distinct from *A. hispanicus* individuals, we wished to examine which variables contribute to the difference. It was hoped

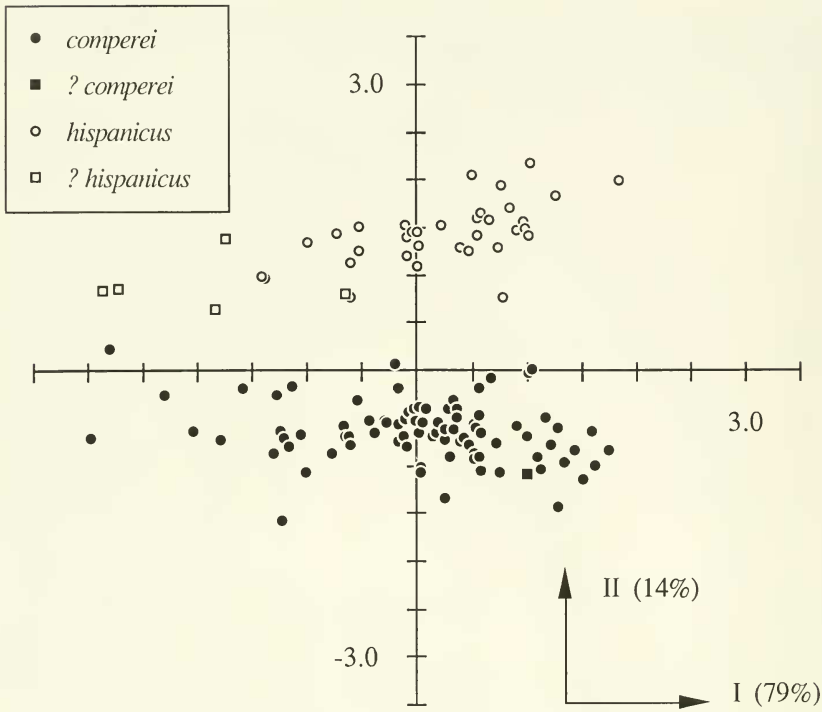


Fig. 8. Observations plotted on the first two principal components computed from the covariance matrix from the untransformed data. The first prinicpal component contains 79% of the sample variance, the second principal component contains 14%.

that the second canonical variate would be constructed in a way that would provide discrimination between *A. hispanicus* and *A. ?hispanicus* individuals.

Once the scores for the original observations on the first two canonical variates were obtained, we constructed 95% prediction regions around the clusters of points for each class using the formulae of Owen and Chmielewski (1985), an application of standard methods (e.g. Johnson and Wichern, 1982). These ellipses have their centers at the group mean for each cluster for each canonical variate and enclose a 95% prediction region in the following sense: if all such prediction regions were drawn, 95% of them would contain each sample point (Owen and Chmielewski, 1985). This technique assumes that the scores for each class on the first two canonical variates have a bivariate normal distribution. We could not test for bivariate normality, but we did test for univariate normality, and in all cases except one, we could not reject the null hypothesis of univariate normality. The one exception was the distribution of scores for the first canonical variate for *comperei*. Inspection of the distribution of *comperei* points on the first two canonical variates revealed one conspicuous outlier. With this

outlier removed the null hypothesis (univariate normality) could not be rejected ($P > 0.15$, Kolmogorov test). For this reason, the 95% confidence ellipse for *comperei* was constructed without the score for the outlier observation.

Results

Principal component analysis.—A projection of the individual specimens on the first two principal components computed from the variance/covariance matrix from the original data is presented as Fig. 8. Together, the first two principal components account for 93.0% of the original variance. The remaining principal components represent increasingly miniscule proportions of the total variance: from 2.1% for the third to 0.03% for the seventeenth. The unitized eigenvectors associated with the first two principal components are shown in Table 3. The elements of each vector have been scaled so that the sum of the squares of all the elements in each vector is unity. Thus, the elements represent weights, and the value for each element squared represents the proportion of variance in the principal component which each variable contributes, assuming that PCA has produced uncorrelated linear transformations of the original variables.

Two distinct clusters of points representing *A. comperei* and *A. hispanicus* individuals were found (Fig. 8). The individual tentatively determined as *A. ?comperei* lies well within the cluster formed by *A. comperei* individuals. The clusters formed by *A. comperei* and *A. hispanicus* are distinct with respect to the second principal component only. Thus, by examining the weights in Table 3 for the second principal component, one can gauge the contribution of individual variables to the location of individuals on this axis. For example, from Fig. 8 and Table 3 it is noted that *A. hispanicus* individuals tend to have longer and narrower forewings with long marginal cilia, a longer infuscate area on the antennal club and more setae in the delta region of the forewing. *Aphytis comperei* individuals tend to have wider forewings and longer ovipositors.

Table 3. Eigenvalues and weights for the first two principal components, computed from the covariance matrix from the untransformed data. The vectors are scaled so that the sum of the squares of the elements in each vector is unity. The rows have been sorted on the elements for the second principal component, from numerically highest to lowest.

Variable or Quantity	PC I	PC II
Eigenvalue	3243.92	555.63
Proportion of Variance	0.794	0.136
14) Setae in delta region, forewing	0.20	0.56
5) Length of infuscate area on club	0.03	0.27
13) Length of LMC on forewing	−0.04	0.26
11) Length of forewing	0.79	0.16
15) Length of middle tibia	0.23	0.12
9) Length of propodeum	0.08	0.10
17) Length of midtibial spur	0.07	0.09
16) Length of basitarsus	0.10	0.07
4) Length of club	0.10	0.03
8) Length of metanotum	0.02	0.01
7) Length of scutellum	0.13	−0.004
1) Length of scape	0.12	−0.01
6) Length of mesoscutum	0.15	−0.02
3) Length of apical funicle segment	0.04	−0.02
2) Length of pedicel	0.04	−0.03
12) Width of forewing	0.30	−0.28
10) Length of ovipositor	0.32	−0.62

The first principal component in Fig. 8 fits some of the criteria (Blackith and Reyment, 1971; Jolicoeur and Mosimann, 1960) commonly used to identify a size vector, or a principal component which reflects primarily variation in overall size of the specimens. The weights for the first principal component in Table 3 are all positive except for character 13. The weights are not, however, of uniform magnitude. The heavily weighted characters (14, 11, 15, 12, and 10) are measurements of comparatively large structures with correspondingly large vari-

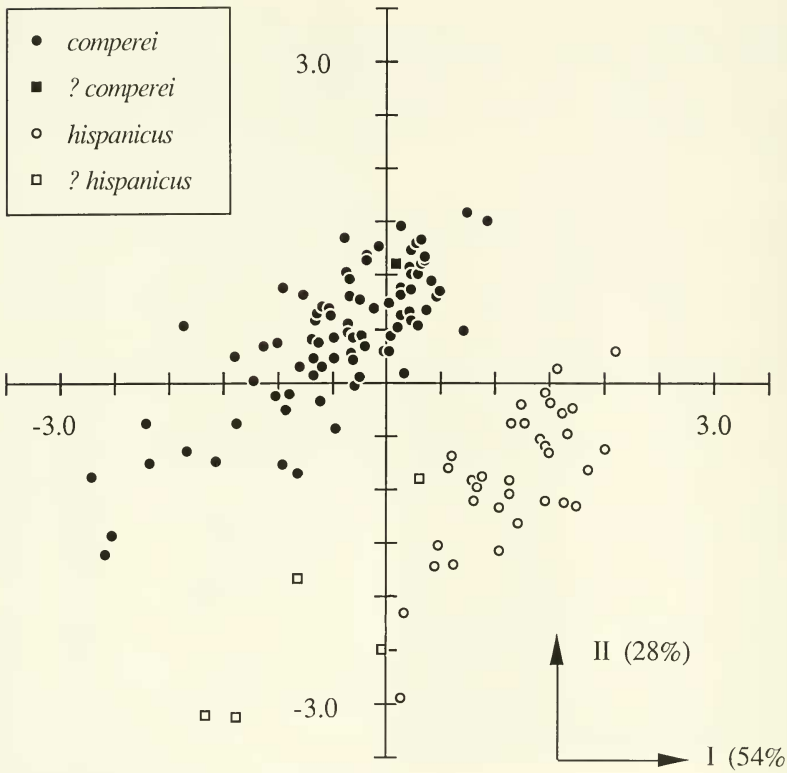


Fig. 9. Observations plotted on the first two principal components computed from the covariance matrix from the log-transformed data. The first principal component contains 54% of the sample variance, the second principal component contains 28%.

ance. We note also that the clusters of points for each species in Fig. 8 are roughly parallel to the first principal component axis, lending further support to the interpretation of this principal component as a size vector. If the first principal component simply approximates overall size, then the five individuals originally determined as *A. ?hispanicus* would appear to be small specimens of *A. hispanicus.*

Figure 9 is a projection of the individuals on the first two principal components computed from a variance/covariance matrix derived from the log-transformed data. This transformation should reduce the overall effect of the large variances associated with variables with numerically large values. Table 4 shows the eigenvalues and eigenvectors for the first two principal components, as discussed above for Table 3. That the tranformation was successful can be seen in Table 4, in that the first principal component now accounts for only 53.8% of the total variance, with a greater proportion, 27.7%, now contained in the second principal component. The remaining principal

components now account for somewhat greater, but still relatively small proportions of the total variance: 4.7% for the third and 0.1% for the seventeenth. The weights for the first principal component (Table 4) are all positive, although not of uniform magnitude, suggesting that the first principal component retains some variance associated with overall size of the specimens.

Again, two distinct clusters of points in Fig. 9 correspond with original determinations of either *A. comperei* or *A. hispanicus* and *A. ?hispanicus*. However, the clusters in Fig. 9 are at oblique angles to two principal component axes, suggesting that now the first principal component expresses variance associated with shape differences in addition to size differences. Also, the inclusion of a point in one or another cluster is now determined by a contribution from both principal components. The contribution of individual variables to the clustering of individuals when projected on the first two principal components can be assessed by examining the relative weights of variables on each component. The length of the infuscate portion of the antennal club is the only variable which makes a strong contribution to both the first and second principal component, accounting for just over 25% of the variance of each. The number of setae in the delta region of the forewing accounts for slightly over 25% of the variance in the first principal component, and the length of the marginal cilia on the forewing for slightly over 25% of the variance represented by the second principal component. Other variables with high weights on the first principal component are the length of the propodeum and the length of the basitarsus. The width of the forewing, length of the pedicel and third funicle segment on the antenna, lengths of the mesoscutum and scutellum, and length of the ovipositor all have relatively high weights on the second principal component.

The *A. ?hispanicus* individuals are again concentrated at one end of the distribution of *A. hispanicus* individuals in Fig. 9. However, the effect now appears to be spread between the first and second principal components. Furthermore, the points now overlap with *A. hispanicus* more with respect to their location on the second principal component. In this case, more than a simple size effect seems to be involved, as the location of points with respect to either component is not simply size related. It appears that these individuals are intermediate with respect to some aspects of morphology, although more similar to *A. hispanicus* than to *A. comperei*. *Aphytis ?hispanicus* was retained as a distinct *a priori* class in the canonical variates analysis for this reason. The *A.?comperei* specimen was treated as a member of the *A. comperei* class for canonical variates analysis because the point representing this specimen fell in the middle of

Table 4. Eigenvalues and weights for the first two principal components, computed from the covariance matrix from the log-transformed data. The vectors are scaled so that the sum of the squares of the elements in each vector is unity.

Variable or Quantity	PC I	PC II
Eigenvalue	0.0341	0.0176
Proportion of Variance	0.538	0.277
1) Length of scape	0.13	0.17
2) Length of pedicel	0.07	0.26
3) Length of apical funicle segment	0.10	0.20
4) Length of club	0.14	0.12
5) Length of infuscate area on club	0.57	−0.51
6) Length of mesoscutum	0.15	0.20
7) Length of scutellum	0.18	0.20
8) Length of metanotum	0.17	0.09
9) Length of propodeum	0.25	0.11
10) Length of ovipositor	0.05	0.26
11) Length of forewing	0.15	0.15
12) Width of forewing	0.11	0.26
13) Length of LMC on forewing	0.09	−0.52
14) Setae in delta region, forewing	0.53	−0.01
15) Length of middle tibia	0.19	0.15
16) Length of basitarsus	0.26	0.18
17) Length of midtibial spur	0.20	0.08

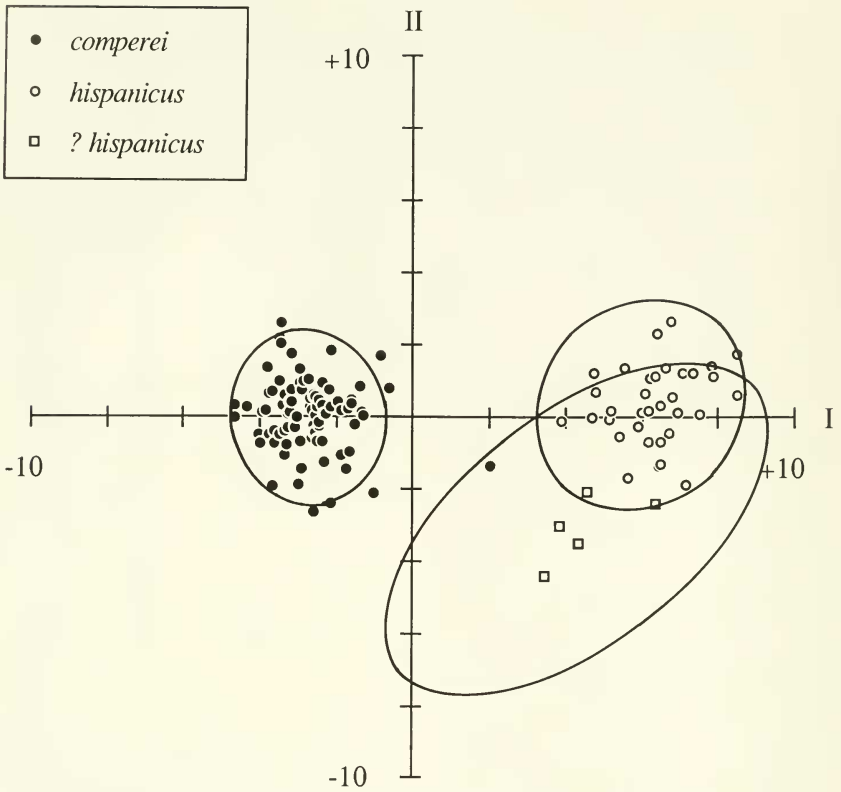


Fig. 10. Observations plotted on the two canonical variate axes. The ellipses around each cluster of points represent 95% prediction regions (Owen and Chmielewski, 1985).

the points for the *A. comperei* specimens when projected against either set of principal components (Figs. 8 and 9).

Canonical variates analysis.—Homogeneity of the covariance matrices for the three classes used in canonical variates analysis was tested using the SAS DISCRIM procedure. The covariance matrix for the *?hispanicus* class was not of full rank due to the small number of observations, and therefore could not be tested against the other two. However, the tests did show that the null hypothesis that the covariance matrices of the *comperei* and *hispanicus* classes were homogeneous could not be rejected (P = 1.000, likelihood ratio test). We assumed that the covariance matrix for the *?hispanicus* class was also homogeneous with the other two, since we had no reason to assume otherwise.

Figure 10 is a plot of the projection of the individuals on the first two canonical variates. The first canonical variate contains 97.6% of the between-groups variance, and since with three groups only two canonical variates can be constructed, the second canonical variate contains 2.4% of the between-groups variance. One would expect

Table 5. Standardized coefficients and total canonical structure for the canonical variates analysis. The standardized coefficients are the amount that the canonical variate score will change for a change in the original variable of one standard deviation. The total canonical structure values are the total-sample correlations between the original variables and the canonical structure scores. The rows have been sorted by the elements of the vector of coefficients for the first canonical variate.

Variable	Standardized Coefficients		Total Canonical Structure	
	CV I	CV II	CV I	CV II
5) Length of club infuscation	1.45	−0.23	0.91	0.15
14) Setae in delta region, forewing	1.17	0.75	0.73	0.57
9) Length of propodeum	0.91	0.40	0.46	0.62
13) Length of LMC on forewing	0.80	−0.39	0.74	−0.35
17) Length of midtibial spur	0.35	0.37	0.45	0.65
15) Length of middle tibia	0.33	−0.45	0.24	0.71
11) Length of forewing	0.18	0.33	0.10	0.76
8) Length of metanotum	0.14	−0.11	0.26	0.40
16) Length of basitarsus	0.09	−0.26	0.30	0.67
4) Length of antennal club	0.09	0.25	0.14	0.66
2) Length of pedicel	−0.02	−0.04	−0.22	0.47
6) Length of mesoscutum	−0.12	−0.67	−0.02	0.68
1) Length of scape	−0.35	0.18	−0.01	0.70
12) Width of forewing	−0.41	0.14	−0.30	0.64
7) Length of scutellum	−0.50	−0.38	0.04	0.66
3) Length apical funicle segment	−0.58	0.83	−0.19	0.88
10) Length of ovipositor	−0.87	0.11	−0.54	0.58

the very tight clusters of points representing the *comperei* and *hispanicus* individuals (Fig. 10), because canonical variates are constructed to maximize between-group covariance relative to within-group covariance. The 95% confidence ellipse for the *comperei* specimens (with one outlier removed, as discussed above) is well separated from the ellipses for both the *hispanicus* and ?*hispanicus* groups, while the ellipses for *hispanicus* and ?*hispanicus* are broadly overlapping. We note also that the ?*hispanicus* ellipse differs in size, shape, and orientation from the other two ellipses, an indication that the covariance matrix for the this class may not be equal (Owen and Chmielewski, 1985).

Clearly, the first canonical variate unambiguously discriminates the *comperei* group from the *hispanicus* plus ?*hispanicus* groups (Fig. 10). The standardized canonical coefficients for the canonical variates (Table 5) are the products of the canonical vector coefficients and the pooled within-group standard deviations for each variable. They represent the amount that the canonical variate score (e.g. in Fig. 10) will change for each change of the original variable by one standard deviation. A large absolute value for a standardized coefficient generally indicates a variable which will be useful in discrimination (but see Campbell and Atchley, 1981, for a discussion of potential problems with this interpretation). The strong positive standardized coefficient scores for the first canonical variate for variables 5, 9, 13, and 14 in Table 5 indicate that *hispanicus* individuals tend to have a longer infuscate area on the antennal club, a longer propodeum, longer marginal cilia on the forewing, and more setae in the delta region of the forewing, respectively. The strong negative score for variable 10 on the first canonical variate indicates that *comperei* individuals have longer ovipositors because the *comperei* cluster in Fig. 10 is in the negative range of the first canonical variate. Weaker negative scores on the first canonical variate in Table 5 for characters 7 and 3 indicate that *comperei* individuals tend

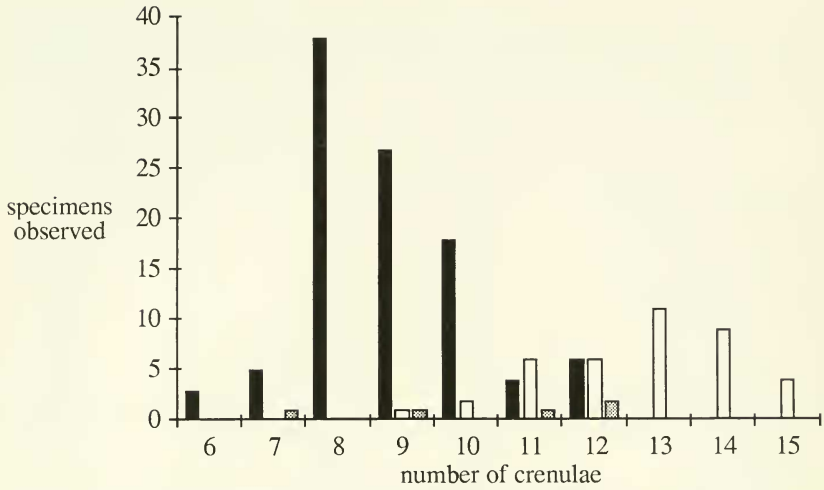


Fig. 11. Number of specimens with observed number of crenulae. (Black bars represent *A. comperei,* white bars represent *A. hispanicus,* shaded bars represent *A. ?hispanicus* specimens.)

to have a longer scutellum and a longer apical funicle segment.

The same pattern is evident in the total canonical structure values in Table 5. These values represent the total-sample correlations between the original variables and the canonical structure scores. As with the standardized coefficients, variables 5, 9, 13, and 14 have strong positive correlations on the first canonical variate. Variable 17, the length of the midtibial spur, also shows a strong positive correlation on the first canonical variate, but its standardized coefficient is relatively low. Variable 10, the length of the ovipositor, has a strong negative correlation on the first canonical variate, with the same implication as discussed above.

Meristic characters.— At least two of the meristic characters for which we recorded data show marked differences between these species. As noted by Rosen and DeBach (1979), *hispanicus* individuals tend to have more crenulae that *comperei* individuals. Figure 11 is a histogram of the number of specimens observed with a particular number of crenulae. Most *comperei* specimens had 8–10 crenulae (see Fig. 4), most *hispanicus* specimens had 11–15 (see Fig. 3), while the *?hispanicus* specimens had an intermediate number. Another useful meristic character is the number of medial setae on the eighth abdominal tergum (Fig. 5). As can be seen in Fig. 12, most *comperei* specimens had two such setae, rarely 1 or 3, while most *hispanicus* specimens had 4, or 5, rarely 3 or 6. The *?hispanicus* specimens were again intermediate with 2 or 3 setae in this location.

Discussion

The *?hispanicus* individuals do not appear to represent a morph distinct from the *hispanicus* individuals. In all plots (Figs. 8, 9, 10) the *?hispanicus* observations cluster at one end or the other of the distributions of *hispanicus* observations. In fact, the *?hispanicus* specimens appear to be simply small *hispanicus* individuals. This can be seen clearly in Fig. 10 and Table 5. The *?hispan-*

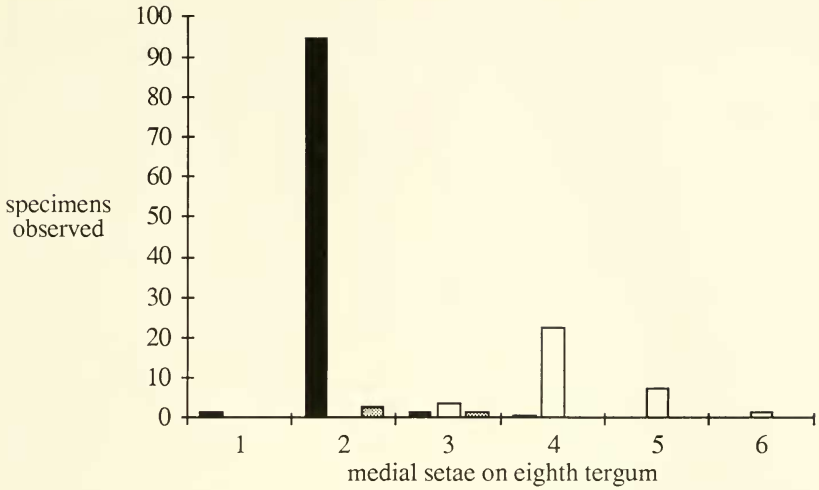


Fig. 12. Number of specimens with observed number of medial setae on the eighth tergum. (Black bars represent *A. comperei,* white bars represent *A. hispanicus,* shaded bars represent *A. ?hispanicus* specimens.)

icus individuals lie below the *hispanicus* cluster in Fig. 10, with virtually all discrimination between these two groups on the second canonical variate. In Table 5, the total canonical structure values show strong positive correlations between the scores on the second canonical variate and the original variables for all variables except 5 and 13. Therefore, since *?hispanicus* individuals tend to have numerically lower (more negative) scores on the second canonical variate, they tend to have numerically smaller values for all variables except 5 and 13. This trend is also apparent in Table 2, in which the means for *?hispanicus* individuals for all variables except 13 are lower than the means for *hispanicus* individuals. The meristic characters are intermediate for the *?hispanicus* class (Figs. 11 and 12), but again, if the *?hispanicus* group consists of small *hispanicus* individuals, they would be expected to have fewer crenulae and fewer medial setae on the eighth tergum.

The *comperei* individuals (except the one outlier) are morphologically distinct from the *hispanicus* specimens (including the *?hispanicus* specimens). In all of the results, the following patterns are consistent. *Aphytis comperei* individuals tend to have a longer ovipositor than *hispanicus* individuals. *Aphytis hispanicus* individuals tend to have a longer infuscate area on the antennal club, more setae in the delta region of the forewing, longer marginal cilia on the forewing, and a longer propodeum than *comperei* individuals, and to some extent, a longer middle tibia with a longer apical spur. Of the diagnostic characters used by DeBach and Rosen (1976) and Rosen and DeBach (1979) (Table 1), most are well supported by our results. However, we did not find *A. comperei* individuals to have consistently shorter clubs (Tables 3–5) as they stated, nor did we find consistently shorter or wider forewings in *A. comperei* (Tables 3–5).

In Table 2 we have tabulated the means, 95% confidence intervals for the means, and observed ranges for the quantitative variables. The means for characters 5, 10, 12, 13, and 14 for *comperei* and *hispanicus* are

well separated (as indicated by the confidence intervals), but the ranges for these variables are strongly overlapping, with the exception of character 5 for which the ranges are contiguous. Therefore, while there is information in many of these characters, any particular variable for a single specimen will not necessarily be discriminating. In fact, the covariation of traits leads to the distinctly different morphologies of *comperei* and *hispanicus*. This points out the dangers in comparison of mean values for characters (e.g. with *t*-tests) or of the ranges of characters in making taxonomic decisions. In this case, comparison of means only would overstate the differences between *comperei* and *hispanicus*, while comparison of ranges only would not reveal trends which do occur in these data. Multivariate techniques such as PCA and CVA explicitly represent the covariation between many characters, thereby providing a method to assess trends which occur in several characters simultaneously. For an excellent discussion of this general problem, see Albrecht (1980).

Conclusions

Our results strongly support the conclusions of Rosen and DeBach (1979) that *A. comperei* and *A. hispanicus* are two distinct, but closely related species. Of course, with thelytokous forms such as these, notions of reproductive isolation do not apply, and the boundaries of species are necessarily arbitrary. We have found rare male specimens in our material referable to one of the two species. The results of Rössler and DeBach (1972, 1973) suggest that the rare males in *Aphytis* species are functional, at least in some species. Therefore, the possibility exists that mating occurs in the field, and that gene flow occurs between clones and even between species as we now recognize them. More likely, however, is the situation observed with uniparental strains of *A. maculicornis* and biparental strains of *A. paramaculicornis* in which laboratory studies have indicated that these forms are completely reproductively isolated (Rosen and DeBach, 1979). The possible role of males in *comperei* and/or *hispanicus* is a matter for further investigation.

Nevertheless, we have found two distinct morphs corresponding almost exactly to the concepts of *A. comperei* and *A. hispanicus* presented by DeBach and Rosen (1976). The results from the principal components analysis are the strongest evidence for the distinctness of the two forms, as this technique does not utilize any *a priori* grouping criteria in dimension reduction. The meristic characters provide further evidence for the discontinuity in the morphologies of the two species. The *A. ?hispanicus* specimens remain somewhat problematic, as both the meristic characters and the multivariate analyses indicate that these individuals are intermediate in form. However, the multivariate analyses also suggest that these specimens are smaller *A. hispanicus* individuals and meristic data are consistent with that hypothesis. We do not, therefore, support recognizing these as a third, distinct morph.

Given that *A. comperei* and *A. hispanicus* are morphologically distinct, how can one best identify individual specimens? The criteria of Rosen and DeBach (1979) (Table 1) will generally be useful, although we would not recommend using the length/width ratios of either the antennal clubs or the forewings. One new meristic character, the number of medial setae on the eighth tergum will be useful in most, but not all cases. However, as noted above, it is the covariation in traits which makes the two species morphologically different, and no single characteristic will provide a reliable criterion for identification in all cases. Discriminant analysis provides a statistical method to identify unknowns to known groups in just such a situation. Discriminant analysis produces a single linear transformation of the original variables to optimally discriminate between a set of predefined groups. Canonical variates analysis, which we have used, is essentially the multi-group exten-

sion of discriminant analysis (Albrecht, 1980), and the special case of CVA of two groups is equivalent to discriminant analysis (Neff and Marcus, 1980). Once a set of diagnostic characters and a discriminant function has been developed for a pair (or group) of cryptic species, the information can be distributed to persons who need to make routine identifications, but who do not have extensive experience with the species and with their subtle diagnostic characters. This person could input a series of measurements for each unknown specimen. The discriminant function would then provide the *a posteriori* probability of an unknown belonging to one of the known species. However, one important caveat exists, all possible species to which the unknown might be referable must be included in the discriminant function for the technique to be valid.

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